RESPIRATORY GRADIENTS IN TUBULARIA[1]

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The presence of a respiratory gradient along the stem of Tubularia has been known for some time (Hyman, 1926; Barth, 1940a). It has been suggested that it is the underlying factor of the regeneration gradient (Barth, 1940b). One can easily find considerable evidence in previous works of various investigators in favor of this idea (Barth, 1940b). For instance, regeneration rates along the stem are roughly proportional to the rate of oxygen consumption and oxygen is an important factor in the rate of regeneration and in determination of the polarity of the stem. However, the evidence so far does not seem critical enough for its establishment.

More recently, Rose and Rose (1941), Miller (1942), and Goldin (1942a) have shown that a certain inhibitor (or inhibitors) of regeneration is produced by the stem. Goldin (1942a, 1942b) and Barth (1944) showed that a ratio involving the concentration of inhibitors and oxygen, $O_2/(\Sigma I)$, somehow determines the rate of regeneration and the polarity of the stem. Thus, another hypothesis suggested by Barth (1944) is that a gradient in the concentration of the inhibitor is the cause of polarity and of the determination of the polarity of the stem. Oxygen merely shifts the threshold of inhibition.

The purpose of the present investigation is to test which of the two hypotheses is more likely. It has been known that the regeneration polarity of Tubularia can be reversed by various methods. If the respiratory gradient is the cause of this polarity, one would expect that when we use one of the methods to reverse the polarity of the stem, the respiratory gradient should reverse its direction before the polarity.

Methods

There are a number of techniques which can be used for reversing the polarity of Tubularia, for instance, differential concentration of oxygen at two cut ends, ligature (Barth, 1938) etc. The method adopted for reversing the polarity in the present paper was ligature. The material used was freshly collected from Atlantic Beach, Long Island, New York City. A number of healthy stems was carefully selected. They were cut into fragments a little more than 10 mm. long. The distal ends of these fragments were then tied individually with a length of hair. Finally they were placed in sea water through which pure oxygen was bubbled, and kept at 15° C. until the initiation of measurements.

For measuring the oxygen uptake, Cartesian diver technique was used. Before the measurement of O_2 uptake, fragments were individually checked under a microscope to see whether they were in healthy condition and whether the primordia had

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been formed. Only the healthy fragments were used in the experiments. Each fragment was then cut into two pieces, the distal and proximal halves of approximately similar size (Tables I, II and III). The respiratory rates of the two pieces derived from the same fragment were then compared. All measurements were made at 25° C. It is very hard in New York City to keep the temperature below this in summer. Although this temperature is higher than normal, the rate of respiration during the period of measurement is constant in all cases. After the measurement, the pieces were carefully taken out of the divers. They were rinsed

TABLE I

Frag. No.	Rate Distal	Rate Proximal
1	0.76	0.60
2	0.83	0.56
3	0.76	1.0
4	0.63	0.53
5	1.0	0.85
$\bar{X}$	0.80	0.71

TABLE I. The respiratory rates of the fragments of the non-regenerating stems. The rate $\times 10^{-3}$ μl./μg. dry wt./hr.

once with distilled water and were subsquently transferred onto the weighing pans. Following this, they were dried overnight in an oven at 120° C. and were then weighed on a quartz torsion balance.

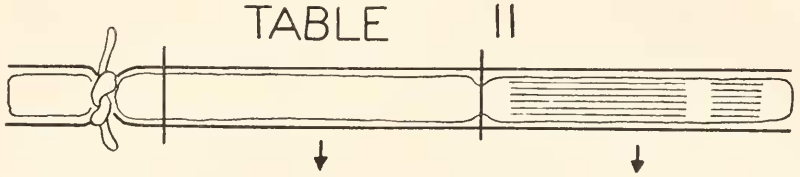
RESULTS

In Table I, there are five experiments made on three-day old fragments that have no primordium. Four fragments show that the distal half has a higher respiratory rate than the proximal portion. One fragment gives the opposite result. On the average the distal half respires more than the proximal. These results agree with the findings of Hyman (1926) and Barth (1940a).

Four experiments have been made on the fragments which have primordia at their proximal ends and no primordia at the distal ends (ligature end). The results are summarized in Table II. From the table one can see that all the distal halves invariably show higher respiratory rates than the proximal ends, although the latter bear primordia.

In Table III, there are five experiments in which the fragments have very well-developed hydranths. The measurements were made three days after the emergence of the hydranth from the perisarc. The pieces for measurements of oxygen uptake were cut in the way shown in the figure of Table III. Only the region that was covered by the old perisarc was used for these measurements. This method of cutting

TABLE II



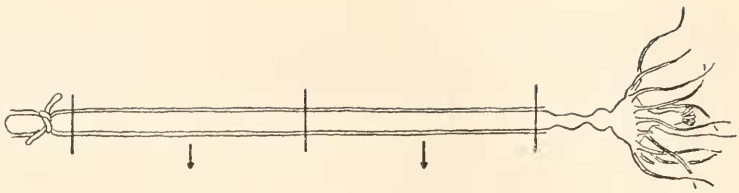
Frag. No.	Rate Distal	Rate Proximal
1	1.8	1.0
2	1.2	0.95
3	1.2	0.68
4	1.6	1.2
$\bar{X}$	1.5	0.96

TABLE II. The respiratory rates of the fragments of the regenerating stems. The rate $\times 10^{-3}$ μl./μg. dry wt./hr.

was employed in order to eliminate possible variation of the thickness of the newly formed perisarc from the old one. From the table we can see that there is no apparent difference between the distal and the proximal piece.

From the results, it is clear that before or during the process of regeneration, there is no apparent change in respiratory gradient. Unfortunately, no running sea water was available. Although sea water was changed every day during the course of investigation, the regenerated hydranth usually dropped off the stem on the third or fourth day after emergence of the hydranth from the perisarc. However, the results on the fragments with well developed hydranths do suggest the tendency of the reversal of the respiratory gradient, since the distal and proximal pieces of such fragments respire at approximately the same rate, three or four days

TABLE III



Frag. No.	Rate Distal	Rate Proximal
1	0.99	0.71
2	0.71	0.93
3	0.62	0.66
4	0.78	0.68
5	0.86	0.86
$\bar{X}$	0.79	0.77

TABLE III. The respiratory rates of the fragments of the polarity-reversed stems. The rate $\times 10^{-3}$ μl./μg. dry wt./hr.

after emergence of hydranth from perisarc. Probably, if one could keep the fragments long enough their respiratory gradient would eventually be reversed.

DISCUSSION

There are two drawbacks concerning the present results. First, the temperature at which the experiments were made may have been too high, even though constant respiratory rates were obtained in all cases during the period of measurement. Second, although measurements of two contiguous pieces of each fragment were made, as shown in the tables, there may nevertheless have been some variation in the thickness of the perisarc, a factor which would affect the total dry weight. In other words, the respiratory gradient could possibly be due to a posterior segment containing more perisarc material and less tissue. Some preliminary experiments indicate that this might well be the case. In the following discussion we shall assume that these two drawbacks are not of great importance. A more extensive study under controlled conditions is of course necessary.

It is possible that techniques which involve keeping an animal at one temperature and measuring oxygen consumption at another may produce a differential acceleration in regions which are compared. If there is any such differential effect of temperature, it does not appear to be important. Barth (1940a) prepared his material at room temperature and measured the oxygen uptake at 18.5° – 19.0° C. His results on the respiratory gradient of the stem are in agreement with the present findings in the experiments on the stems with or without primordia.

A number of investigators have demonstrated the presence of an inhibitor of regeneration. Certain experiments have indicated that the inhibitor substance is a fairly diffusible one (Rose and Rose, 1941; Goldin, 1942a). Barth (1940a) found that the O_2 consumption of regenerating stems is no greater than the O_2 consumption of resting, non-regenerating stems. Thus, stems which were prevented from regenerating by means of ligatures at the ends consumed as much oxygen as stems which regenerated freely. These facts suggest two things: (1) the inhibitor of regeneration is either a weak respiratory inhibitor or an inhibitor not affecting respiration at all; and (2) ligatures have no or very little effect on the respiration of the stem.

It was shown in the present investigation that prior to, or during the process of regeneration, the original respiratory gradient is maintained. In view of the conclusions in the preceding paragraph, these findings really represent what had been going on in the stems before removal of the ligature and cutting. Therefore one may say from the present investigation that the formation of the hydranth is independent of the respiratory gradient. The hydranth is not always formed at the place where the highest respiratory activity exists. If a respiratory gradient were involved in regeneration, one would expect, at least during the time of regeneration, that the primordium would show a higher respiratory activity than the distal segment in the present experiments. Since the presence of an inhibitor of regeneration in the stem has been demonstrated and it has also been found that oxygen plays a very important role in regeneration, it seems most likely that the position of a future hydranth in a regenerating stem is determined by the balance between the concentration of the inhibitor of regeneration and the concentration of oxygen.

Finally, the present findings seem to suggest that the respiratory gradient of the stem is the result of the presence or the activity of a well developed hydranth.

Summary

It was found that during regeneration of the hydranth, the original respiratory gradient of the stem is maintained. Only after the hydranth has formed has the stem a tendency to reverse its original respiratory gradient.

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